

## STRUCTURE AND VARIATION IN SPORES OF THELYPTERIS PALUSTRIS

ALICE F. TRYON

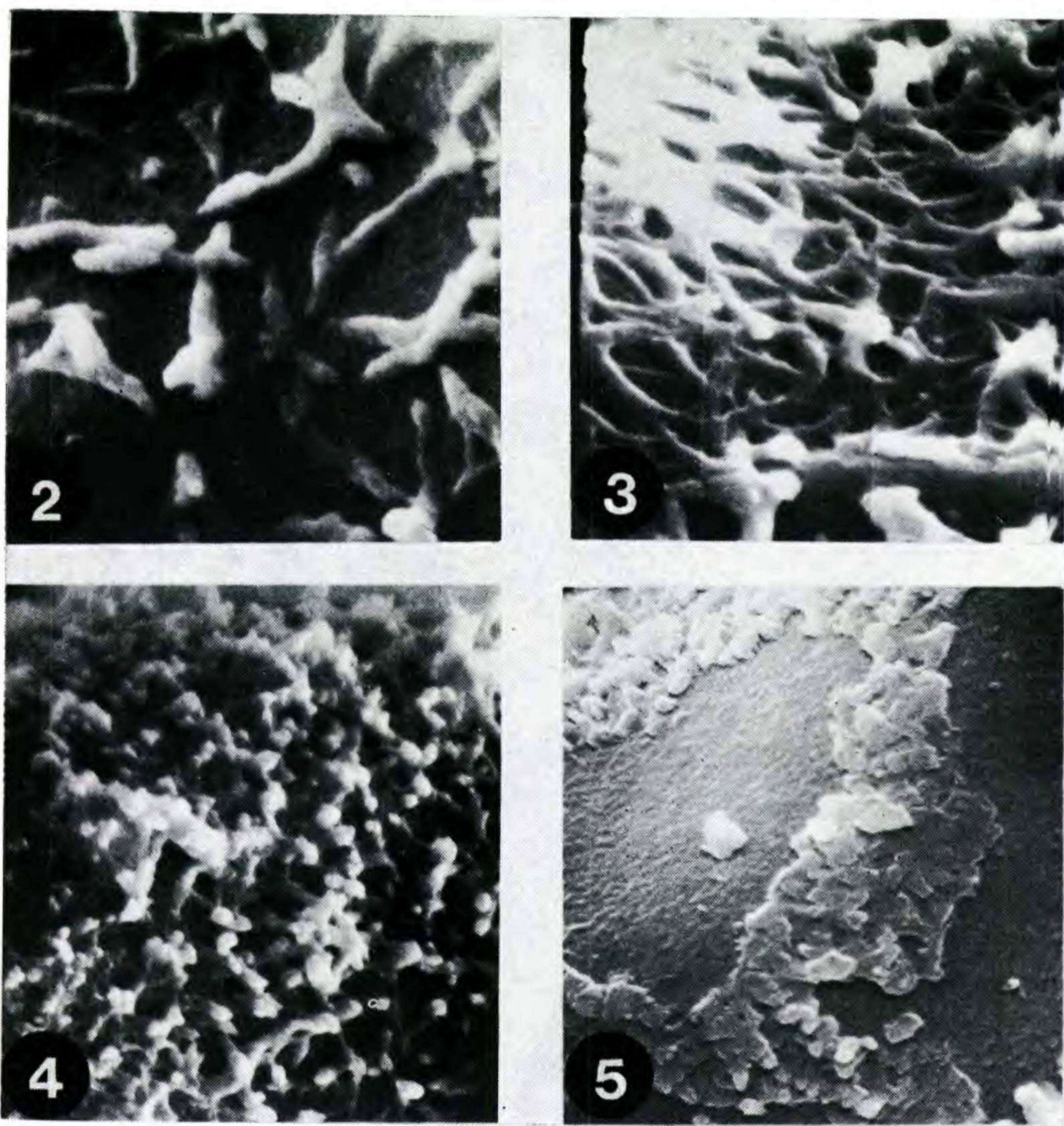
The Marsh Fern is abundant in New England growing in wet, usually open places, often bordering Red Maple woods. It frequents wet sites such as open bogs or swampy stream banks, over a wide geographic range — across Eurasia, in India and Burma, in Africa from the Cameroons and Kenya to Cape Province in the Republic of South Africa, in New Zealand, New Guinea and Sumatra, as well as North America (Fig. 1). Spores from plants of different geographical regions, examined with the scanning electron microscope, show exceptional differences in perine sculpture. The usual variation in the perine layer of spores of *Thelypteris palustris* is assessed in relation to spore development and to the geographical varieties. In other species of ferns and often within genera the spores are relatively similar in sculpture. Indeed, the uniformity by which these patterns are usually replicated demonstrates remarkably regulated morphogenetic systems.

The four varieties of *Thelypteris palustris* Schott, designated by Fernald (1929), included var. *pubescens* (Lawson) Fernald of northeastern North America and eastern Asia; var. *Haleana* Fernald of the southeastern United States and Bermuda; var. *palustris* (Fernald's var. *typica*) of Eurasia; and var. *squamigera* (Schlecht.) Weatherby of Africa, India, Burma, New Zealand and more recently reported from Sumatra and New Guinea. Treatment of these as a single species, as noted by Morton (1967), now requires the application of an earlier name, *Thelypteris confluens* (Thunb.) Morton, based upon a South African collection. In a comparative survey of *Thelypteris* with three other genera, Holttum, Sen and Mitra (1970) note that var. *squamigera* might be better treated as a separate species, and that the status of Fernald's other varieties needs further consideration. The well-known name *Thelypteris palustris* is used here, pending definitive systematic studies of greater scope and further clarification of names.

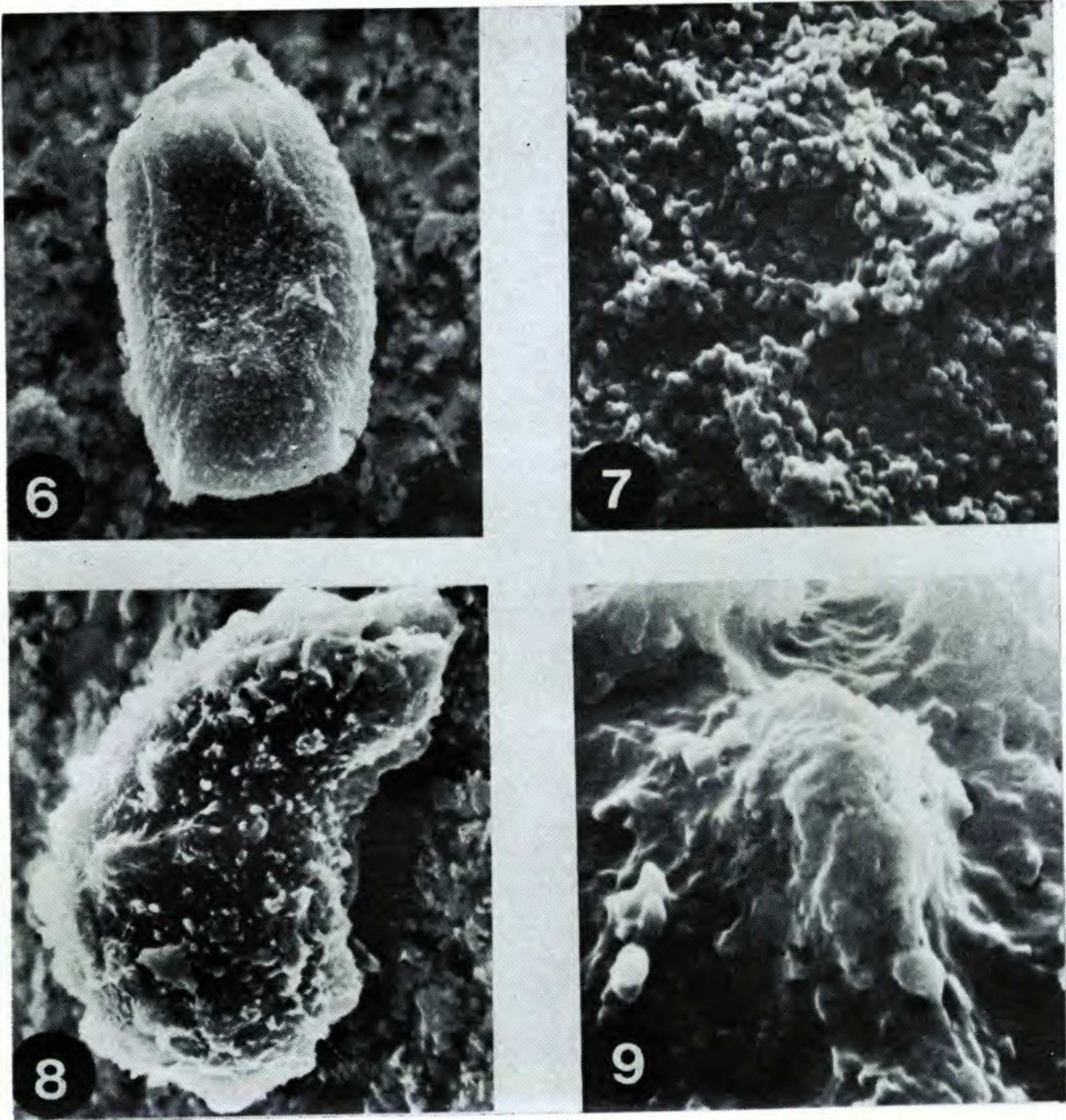
## MATERIALS AND METHODS

The collections of *Thelypteris palustris* in the Gray Herbarium have been the principal source for this study; some materials from the United States National Herbarium (Smithsonian Institution) have also been used. Citations of collections are included where they are appropriate and in captions for figures. Plants of var. *pubescens* from Maine, New York, Wisconsin, and population samples from several localities in eastern Massachusetts have been used for study of stages in spore wall development. These specimens were grown at the same site, along with native plants, in Lexington, Massachusetts. The native Lexington material has been most extensively studied and a voucher for this (*A. F. Tryon* 70-18) as well as the other transplanted specimens, are deposited in the Gray Herbarium.

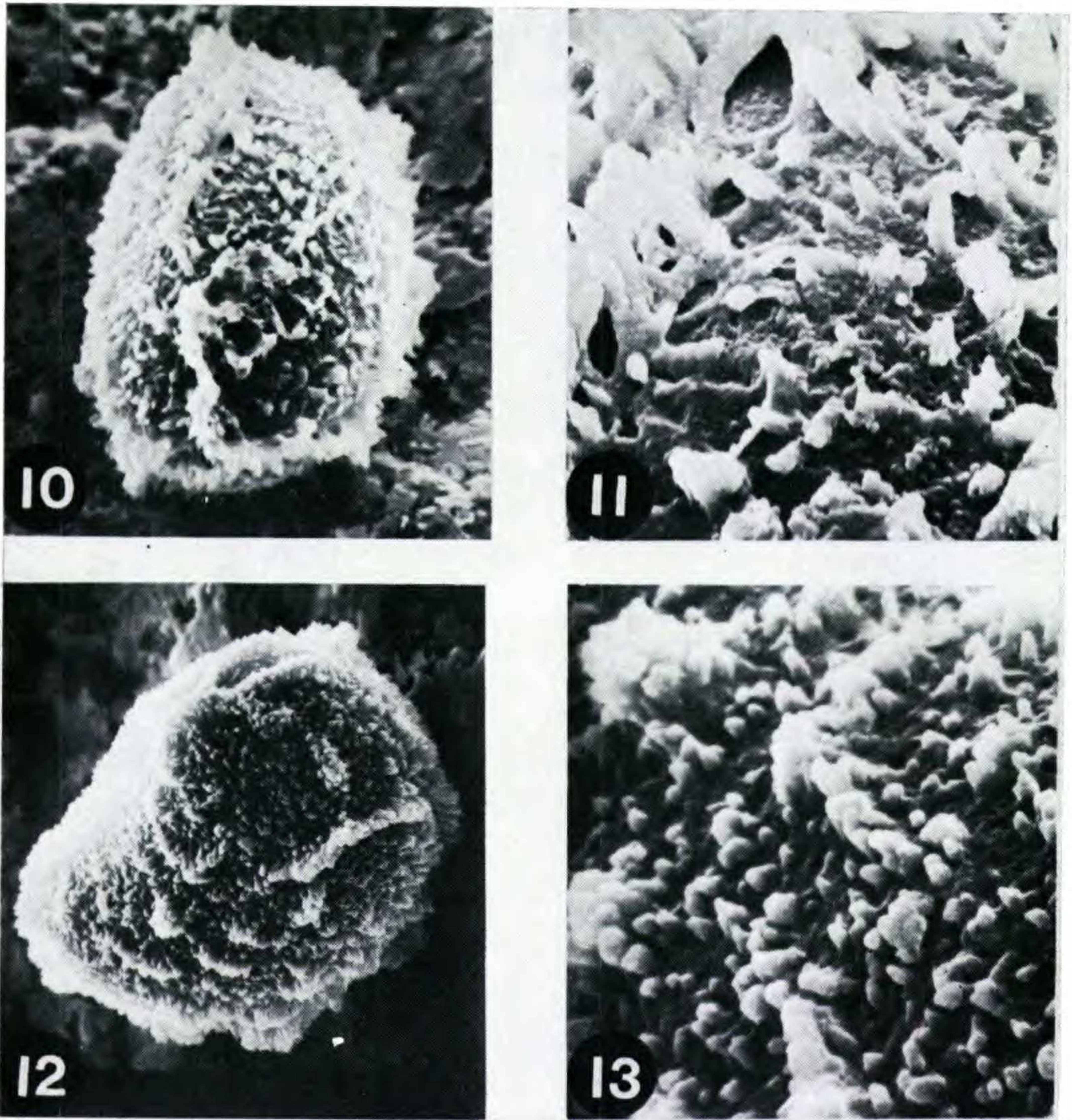
The descriptions of spores of each variety are drawn from specimens studied with both light and scanning electron microscopes. Preparation for the latter were made from non-acetolized spores from herbarium specimens and were prepared by two methods. Those done at the University of Illinois, Chicago Circle, in the laboratory of Dr. Thomas N. Taylor are illustrated in Figs. 6, 8, 9, 10-13, 16, 17, 19, 20. They were gathered in distilled water, dried and fixed on standard aluminum specimen stubs with a thin layer of silver conductive paint. They were coated with evaporated gold while rotating the sample to insure even deposition. Other spores, illustrated in Figs. 2-5, 7, 14, 15, 18, 21, 22, were gathered with a dry brush and transferred onto double-stick Scotch tape fixed to the aluminum stubs. These specimens were undercoated with carbon prior to coating with an evaporated alloy of palladium-gold while on a rotating and tilting platten. The original magnifications of the photographs of whole spores were between 1,000 and 2,000 times; the details were photographed between 5,000 and 10,000. The plates are reduced approximately one-third. Spore size is regarded as approximate because of differences in magnification factors on the different instruments. Some artifacts are conspicuous at the



Figs. 2-5. Surface of spores of *Thelypteris palustris* var. *pubescens*, Pammel 579, Iowa (GH); Figs. 2, 3. Partial deposition of perine layer,  $\times 6600$ ; Fig. 4. Complete deposition of perine layer,  $\times 3300$ ; Fig. 5. Partially eroded perine material exposing smoother exine layer,  $\times 3300$ .

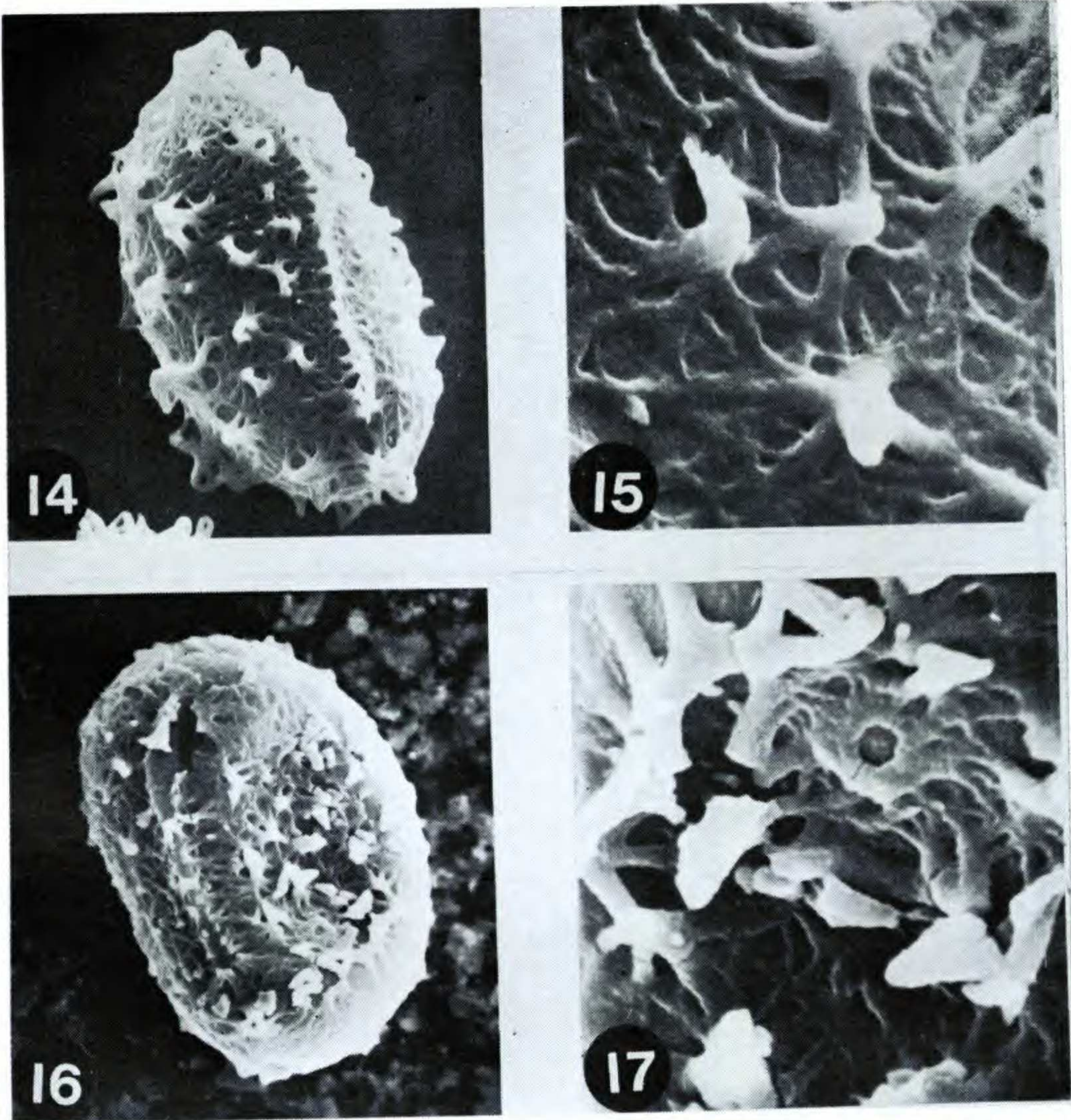


Figs. 6-9. Spores of *Thelypteris palustris* var. *pubescens*: Figs. 6, 7, A. F. Tryon 70-18, Lexington, Massachusetts (GH); Fig. 6. Spore with verrucate perine layer (the exine layer is exposed in the break, at the top of the spore)  $\times$  860; Fig. 7. Papillate surface of the perine layer,  $\times$  4400; Figs. 8, 9. Clements 293b, Nebraska (GH); Fig. 8. Spore with irregularly sculptured perine layer,  $\times$  1000; Fig. 9. Papillate and coarser projections,  $\times$  3500.

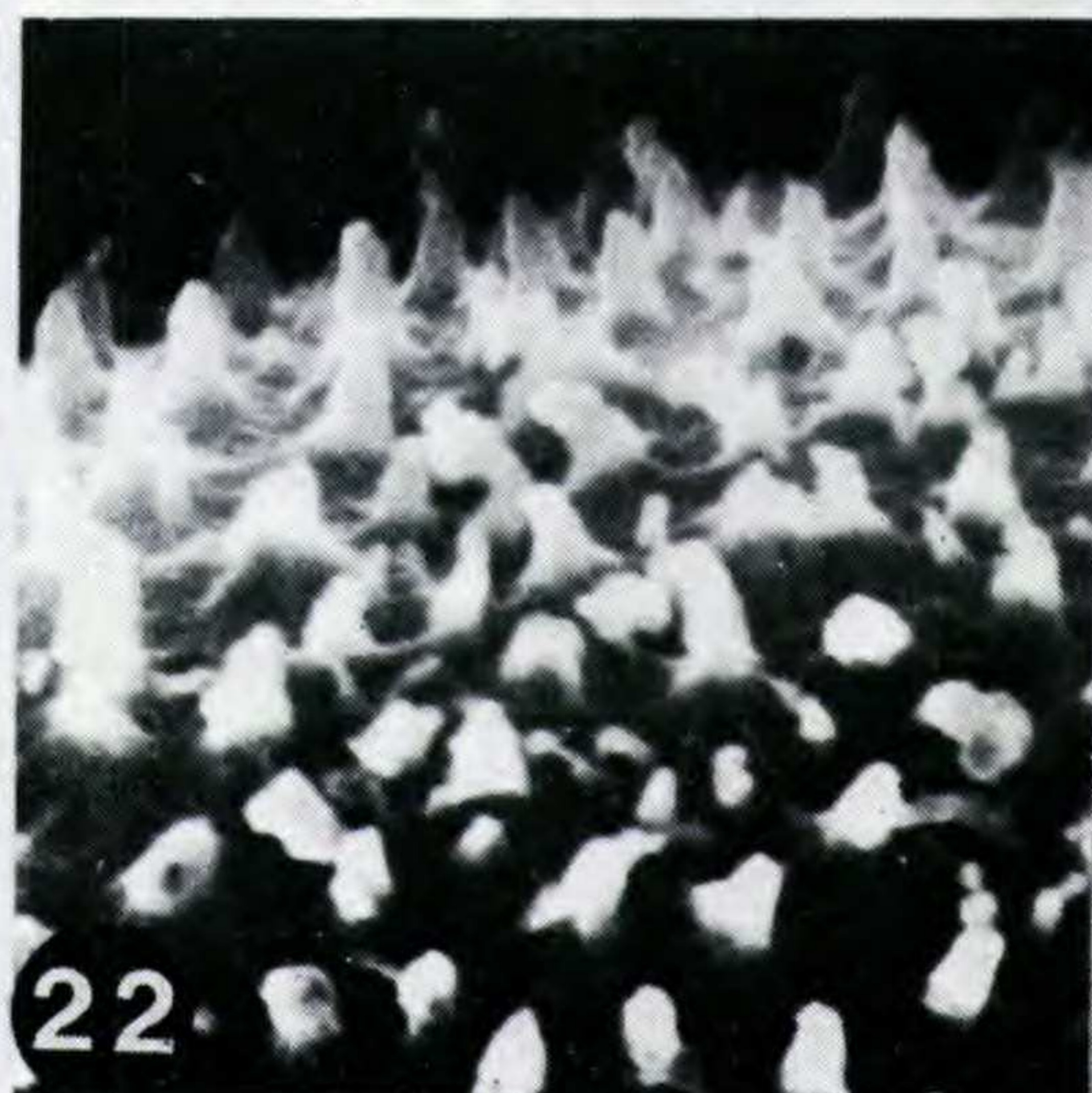
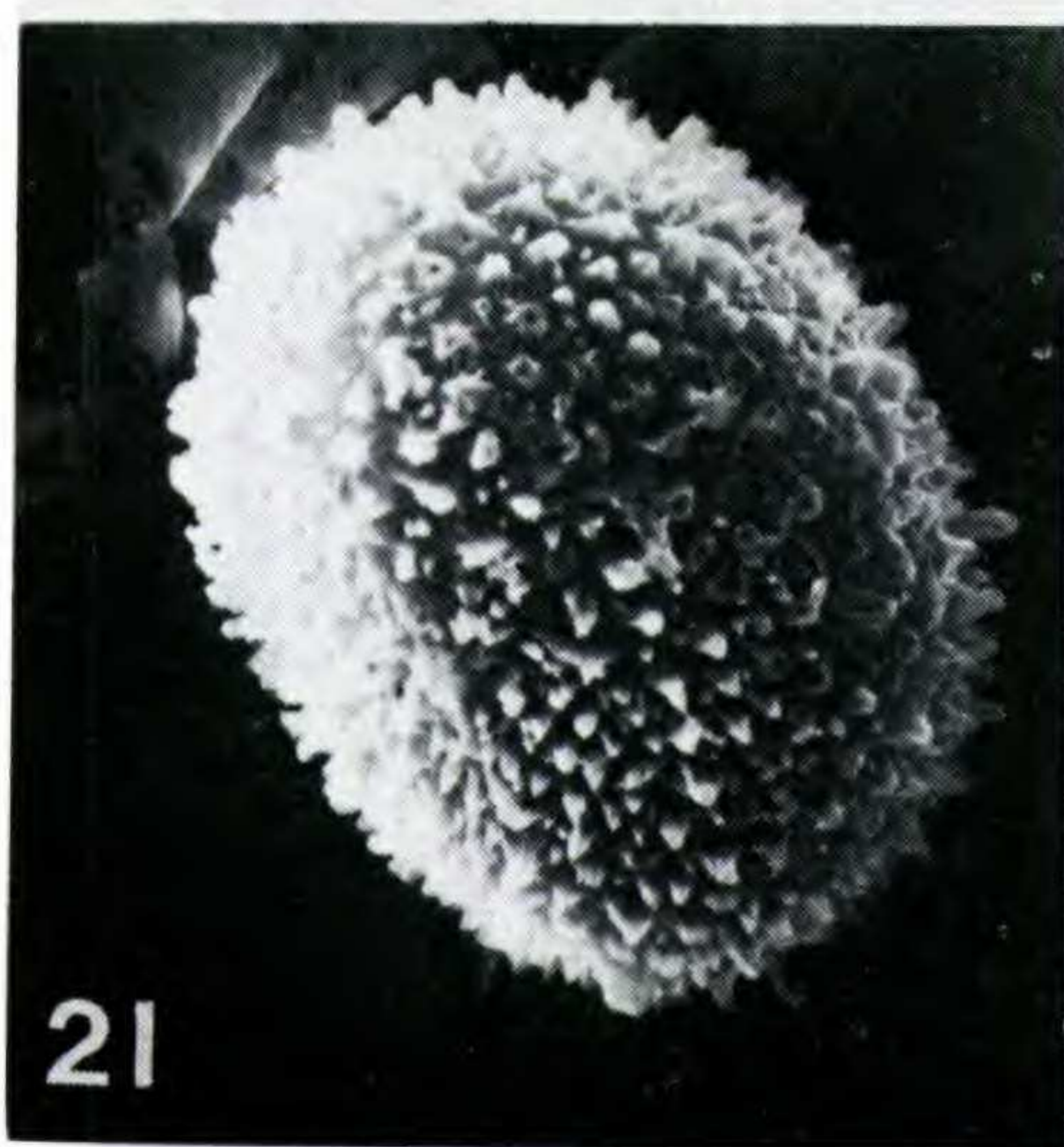
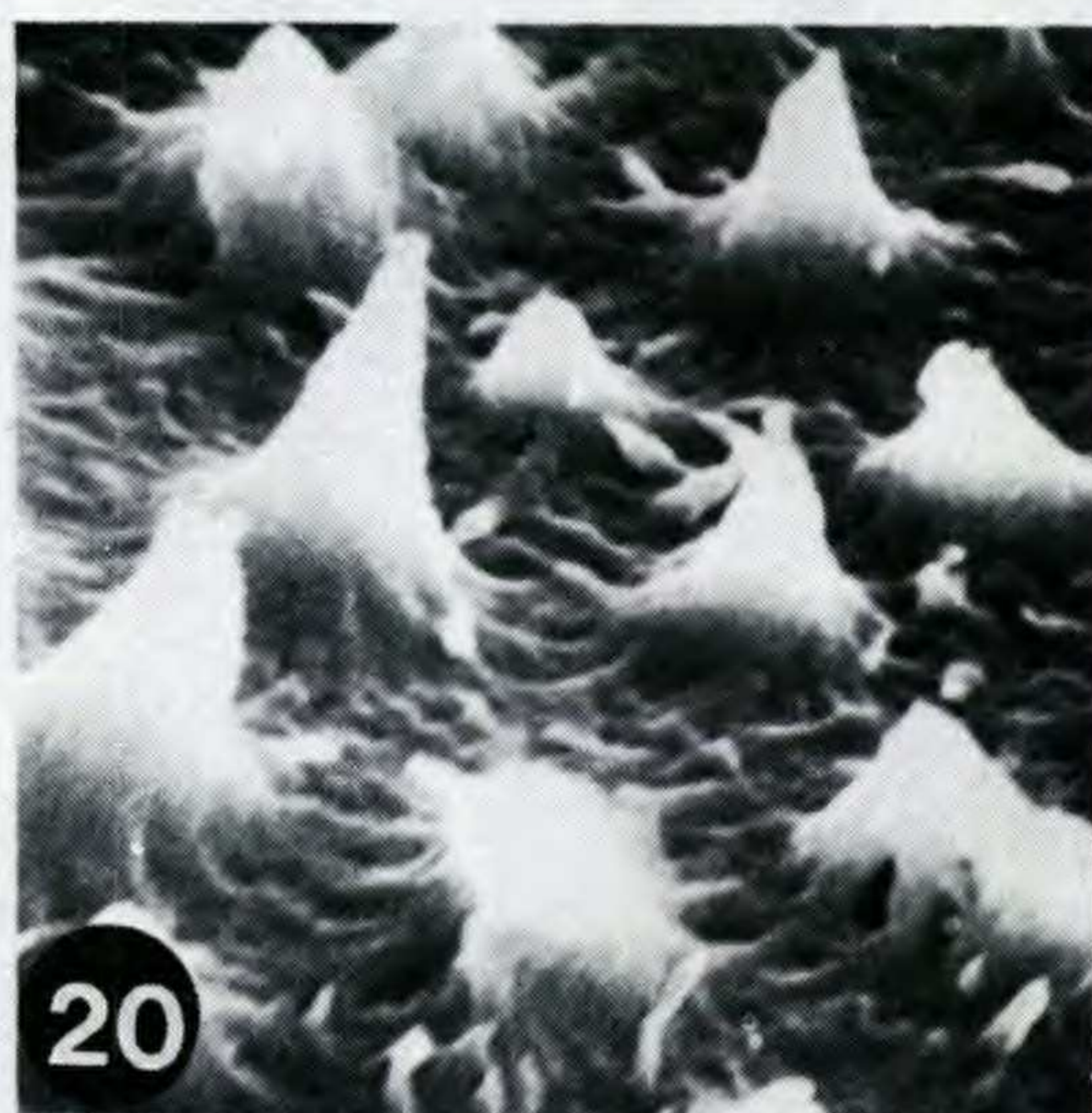
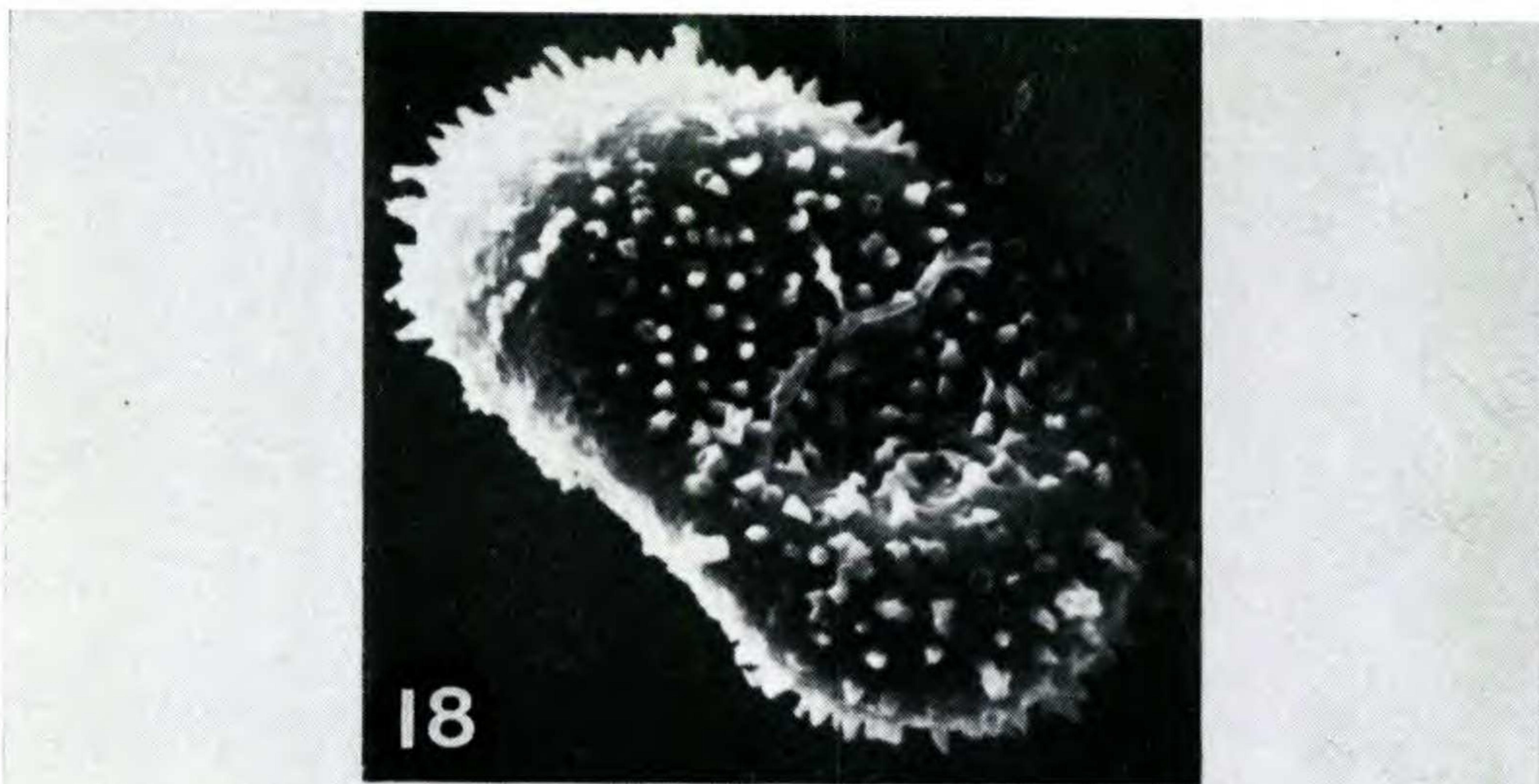


Figs. 10, 11. Spore of *Thelypteris palustris* var. *pubescens*, Furse, in 1957, Hondo, Japan (GH); Fig. 10. Spore with basal reticulum, papillae and higher ridges,  $\times 650$ ; Fig. 11. Papillae, areolae and basal reticulum,  $\times 3500$ .

Figs. 12, 13. Spore of *Thelypteris palustris* var. *Haleana*, Bloomquist & Correll 6136, Florida (GH); Fig. 12. Spore with papillate perine layer and more prominent ridges,  $\times 1000$ ; Fig. 13. Papillae and short ridges,  $\times 3600$ .



Figs. 14-17. Spores of *Thelypteris palustris* var. *palustris*: Figs. 14, 15. Pterid. Exsicc. Walter-Calle 42, Estonia (GH); Fig. 14. Spore with reticulate perine layer and projecting loops,  $\times 860$ ; Fig. 15. Basal reticulum and projecting loops,  $\times 4400$ ; Figs. 16, 17. Exsicc. Petrak, 702, Austria (GH); Fig. 16. Spore with reticulate perine layer and acuminate or looped projections (the commissural ridge is to the left of the center of the spore),  $\times 1000$ ; Fig. 17. Reticulum and echinate projections (several of these broken),  $\times 4400$ .



high magnification, such as the broken processes near the top of the spore and ridge across the center of the New Zealand specimen in Fig. 18. The heavy coating at the base of the spore from Kashmir (Fig. 19) obscures the perine. The age and maturity of specimens are more critical factors in comparing the structural details of the perine layers. The photographs (Figs. 2-5) of portions of different spores of the same collection, at high magnification, show variations in perine deposition. Loss of the outer layer over much of the spore surface exposing the underlying exine layer in Fig. 5, seems to be a natural occurrence in var. *pubescens*. Erosion of this type may account for the frequent damage or loss of the perine layer in the process of acetolizing spores. The high percentage of smooth fern spores reported in studies of fossil material is probably also related to this condition.

A larger number of samples of material fixed in 85 percent lactic acid for two or more hours were examined with the light microscope. Immature sporangia, sporocytes and meiotic cells were also examined with the light microscope from material stained in aceto-carmin following fixation in 3:1 alcohol and acetic acid.

#### ACKNOWLEDGEMENTS

I am indebted to Dr. Thomas N. Taylor, University of Illinois, Chicago Circle and Robert G. Stolze, Field Museum, Chicago, for the SEM photographs noted above; and to F. N. Molea and G. R. Pierce, McKay Laboratory, Harvard University, for training on the Harvard instrument. For constructive comments on the manuscript, I am grateful to David S. Barrington, Umesh C. Banerjee, and especially to Rolla Tryon.

---

Figs. 18-22. Spores of *Thelypteris palustris* var. *squamigera*: Fig. 18. Kirk, Bay of Plenty, North Island, New Zealand (US), spore with diffuse echinate perine layer,  $\times 1200$ ; Figs. 19, 20. Thompson, Kashmir (GH); Fig. 19. Spore with echinate perine layer (looped projection at top right),  $\times 860$ ; Fig. 20. Echinate sculpture and basal reticulum,  $\times 4400$ ; Figs. 21, 22. Drummond & Hemsley 2187, Tanganyika (US), Fig. 21. Densely echinate spore,  $\times 1000$ ; Fig. 22. Echinate perine layer (basal reticulum at left of center),  $\times 3200$ .



## SPORE DEVELOPMENT AND STRUCTURE

Plants of var. *pubescens* studied, from Wisconsin, Maine and several from eastern Massachusetts, uniformly have 35 pairs of chromosomes at meiosis. Nuclear division precedes formation of the cell wall, resulting in four haploid sets of chromosomes within a single cell. At this stage, structural evidence of new walls or of cleavage was not observed. The walls which will partition the four cells in the tetrad become apparent at the same time. As the spores enlarge in the sporangium, in each tetrad they are aligned in a cruciform arrangement with the long axes of the pairs at right angles to each other. The convex surfaces of the spores become strongly curved as the cells enlarge and a gap develops in the center of the tetrad. When the spores are disassociated from the tetrad, a complement of 64 spores fills each of the sporangia. At this stage the spore walls are thick, and without special staining show dense granular organelles and plastids within the protoplast. Perine deposition is not evident on these thick walls, thus it appears that this material may be formed relatively late in sporogenesis. There is considerable variation in spore size within a sporangium in var. *pubescens* as some cells may be up to one-third larger than other well-developed ones.

In *Thelypteris*, the exine layer of the spore wall is sparsely textured in comparison with the outer perine layer, as shown in Fig. 5 and also at the top of the spore in Fig. 6 where the perine layer is broken. The gap between these layers clearly differentiates exine and perine stratification which composes the spore wall.

The work of Pettitt (1966) on ultra structure of *Asplenium Adiantum-nigrum* spores is helpful in interpreting spore wall structure in *Thelypteris*. The perine in *Asplenium* was shown to consist of four layers, two of them forming a raised sculptural pattern similar to that in *Thelypteris*. Two additional layers of perine in *Asplenium* are composed of more homogeneous material and conform to the contour of the subtending exine layer. This stratification suggests that the perine layer may be formed by a more

complex process than gelation, as discussed by Harris (1955). The structure of the perine layer in some species of *Bolbitis* has been examined by Hennipman (1970) and in this genus it was shown to consist of two well-defined layers. Sections through the sporangia in *Bolbitis*, with approximately mature spores, showed that the interstitial region between the spores was filled by a definite reticulum which was considered to form the outer perine layer.

The distinctive forms of perine sculpture shown in the geographic varieties of *Thelypteris palustris* suggest that the structure of this layer can be highly modified within a relatively closely related group. The series of photographs (Figs. 2-5) illustrates different development of the perine material in spores of a single collection of var. *pubescens*. The basis of some differences in the perine layers are shown at high magnification. The denser deposition in Fig. 4 represents additional material superimposed upon the coarser reticulated structure in Fig. 3. Evidence of similar subtending layers is often apparent in spores with outer verrucate or granulate perine layers, as shown in Figs. 10 and 11 of var. *pubescens*. The reticulate structure, shown in Fig. 3, is similar to that characterizing the outer perine layers in specimens of var. *palustris* from Europe (Figs. 14, 15) and of var. *squamigera* from Kashmir (Fig. 20). The verrucate material characteristic of the spores of var. *pubescens* and var. *Haleana* appears to represent an additional perine layer and these spores may represent a more derived form.

The marked differences in perine structure in the two species of *Bolbitis* studied by Hennipman were thought to reflect genetical differences which affect the interaction of haploid and diploid materials during spore formation. Differences shown in the perine layer of the varieties of *Thelypteris palustris* may represent structural adaptations of the spores to different ecological or climatic conditions in the evolution of these plants over their broad geographic range.

Studies on spore morphology at the ultra structural level, such as those by Heslop-Harrison (1968) on developmental

sequences in pollen, are critically needed for fern spores. The work on development and stratigraphy of pollen is applicable to spores. However, fern spores have a single nucleus and represent a simpler condition in comparison to pollen in which the microgametophyte has developed. In ferns, deposition of the external spore sculpture involves a tapetal periplasmodium, while in pollen of many higher plants a rigid cellular tapetum functions in wall formation. There are also differences in external forces which affect these structures. Fern spores are universally distributed by air, and there are no secondary agents such as insects, which are often involved in transport and influence the form of pollen in angiosperms.

#### MATURE SPORES OF THE VARIETIES OF THELYPTERIS PALUSTRIS

Spores of var. *pubescens* from Lexington, Massachusetts have a uniformly granulate or verrucate perine layer (Figs. 6, 7). The texture of this outer perine layer is considerably rougher than the subtending exine layer of the wall which can be seen at the top part of the spore (Fig. 6). In this variety the spores usually have a homogeneous perine layer with short, uniform projections. Some specimens, especially from the western part of the range, from Nebraska, Iowa and Illinois, have more irregular perine structure as seen in Fig. 8. At higher magnification (Fig. 9) the form of this perine is rougher but consists of papillate projections similar to those of spores from the eastern part of the range.

Spores of specimens of var. *pubescens* from Japan (Figs. 10, 11) usually have more prominent perine sculpture than the American material. These spores resemble the more strongly sculptured ones of the plants from the southern United States noted below.

Spores of var. *Haleana*, from Florida, have low, verrucate sculpture and some more prominent ridges as seen in Figs. 12, 13. Specimens from Bermuda have similar spores. The dense, relatively low, granulate or verrucate perine layers in this and the other mainly American variety are

basically similar, in contrast to those of the other two varieties of the Old World.

Spores of var. *palustris* from Europe, as shown by specimens from Estonia and Austria, have a strongly echinate perine layer, as shown in Figs. 14-17. The basal reticulum in these consists of prominent ridges encompassing large, irregular areolae, and projecting peaks or loops. The loops, shown at higher magnifications in Fig. 15, arise from the reticulum, and suggest that during the formation of this material a force is exerted on the strands, pulling them from the spore surface. The specimen from Austria has more prominently echinate perine and the detail of this (Fig. 17) shows several projections which are broken. The commissural ridge, at the left in the photograph of the whole spore (Fig. 16), appears to interrupt the reticulum. Spores of this variety from Finland (called *Lastrea Thelypteris*) were illustrated and described as having echinate perine by P. Sorsa (1964).

Spores of var. *squamigera* have prominently echinate perine as illustrated (Figs. 18-22). Loops may also occur, as shown in Fig. 19, and the lower reticulate layer with areolae and ridges is similar to that in spores of var. *palustris*. The spore surface of the Kashmir specimen, at higher magnification (Fig. 20), shows an aggregation of strands coalescent at the base of the projections. African specimens of this variety usually have denser and often more delicate projections as in Figs. 21, 22. Those from New Zealand have similar, but coarser and more diffuse, echinate sculpture (Fig. 18). New Zealand spores were illustrated by Harris (1955) and described as having a fairly regular papillate perine.

#### CHARACTERS OF THE VARIETIES OF THELYPTERIS PALUSTRIS

The concept of four varieties in *Thelypteris palustris*, proposed by Fernald, has been a useful one to indicate the geographic differentiation within the species. Exceptional variation in spore sculpture suggests that relationships may be more complex than shown in this classification. The principal characters used by Fernald to distinguish the

varieties were surveyed to determine possible correlation of these with the spores.

The condition of the veins of the fertile leaf was a major feature used by Fernald to distinguish the varieties. His comparison of venation was based on the number of simple or forked veins per segment. He reported veins as mostly forked in var. *palustris* and var. *Haleana* and simple in var. *pubescens* and var. *squamigera*. The present survey confirms these general trends. It also shows that a relationship exists between the condition of the veins and the size and degree of modification of the fertile lamina. Fertile leaves in var. *pubescens* tend to have simple veins and the leaves are more strongly dimorphic than in var. *Haleana* in which the veins are mostly forked. Several collections have been noted within the range of var. *pubescens* that have large fertile leaves with broad pinnae and with many forked veins (*Pammel* 579, Iowa; *Huett* from Ottawa, and *Geyer* from Beardstown, in Illinois; *Braun* from Kentucky). Generally, the Eurasian var. *palustris* has relatively large, fertile leaves with broad pinnae. The veins are usually forked, although some specimens with somewhat dimorphic leaves may have simple veins. Thus, fertile and sterile leaves in var. *palustris* and var. *Haleana* are more uniform in size and the veins are most commonly forked. Fertile leaves are modified in var. *pubescens* and var. *squamigera* and in these the veins are mainly simple, in contrast to the forked veins in sterile leaves.

Capitate glands on the indusia most readily characterize var. *palustris*. However, these indusial glands occur in some material of each variety. They are especially well-developed in collections of var. *Haleana* (*Correll* 5907) from Florida. In var. *pubescens*, specimens with glandular indusia such as *Clements*, Nebraska, are mostly from the western part of the range. Similar glands are abundant on the capsule faces of the sporangia in both varieties in eastern North America and are absent or rare on sporangia of the Old World plants.

Scales of the costa are particularly conspicuous in var. *squamigera* and provide the most useful character in dif-

ferentiating this variety. However, scales occur in some collections of each of the varieties. They are especially well-developed in some collections of var. *pubescens* from the western part of the United States (*Clements*, Nebraska). These scales are most abundant on the young leaves and may vary considerably in size. They persist on older leaves in var. *squamigera* and are more or less cauducous in the other varieties.

Some correlations are shown between other characters and variants in the spores, as noted above. However, the two basic forms of the perine layer — echinate in spores of plants from the Old World and verrucate in specimens mainly in North America — suggest new relationships within the species.

#### GEOGRAPHY

The present disjunct geographic distribution implies that the species may be a relatively old one. On the basis of its geographical area and adaptability to swamp life, Ching (1963) considered it archaic and a relict, possibly of the early Tertiary. Fernald regarded the species as having temperate rather than boreal affinities and suggested a tropical or subtropical origin. A boreal origin seems unlikely, as does a temperate one, during Pleistocene time, because of extensive glaciation over these regions. A tropical or subtropical origin during pre-Pleistocene time is regarded as more feasible. The New World tropics can be eliminated as a possible center since this region is remote from the current range of the species.

Continental southeast Asia is proposed as a possible early geographic center of the species, since three of the varieties now occur peripheral to this area, and the morphological diversity in the species is found largely among them. The arrows on the map (Fig. 1) indicate possible lines of geographic and evolutionary radiation from a southeast Asian center. The present range, which did not necessarily expand simultaneously in all directions, has undoubtedly been influenced by Pleistocene and post-Pleistocene environmental changes. The greater geographic disjunction in var.

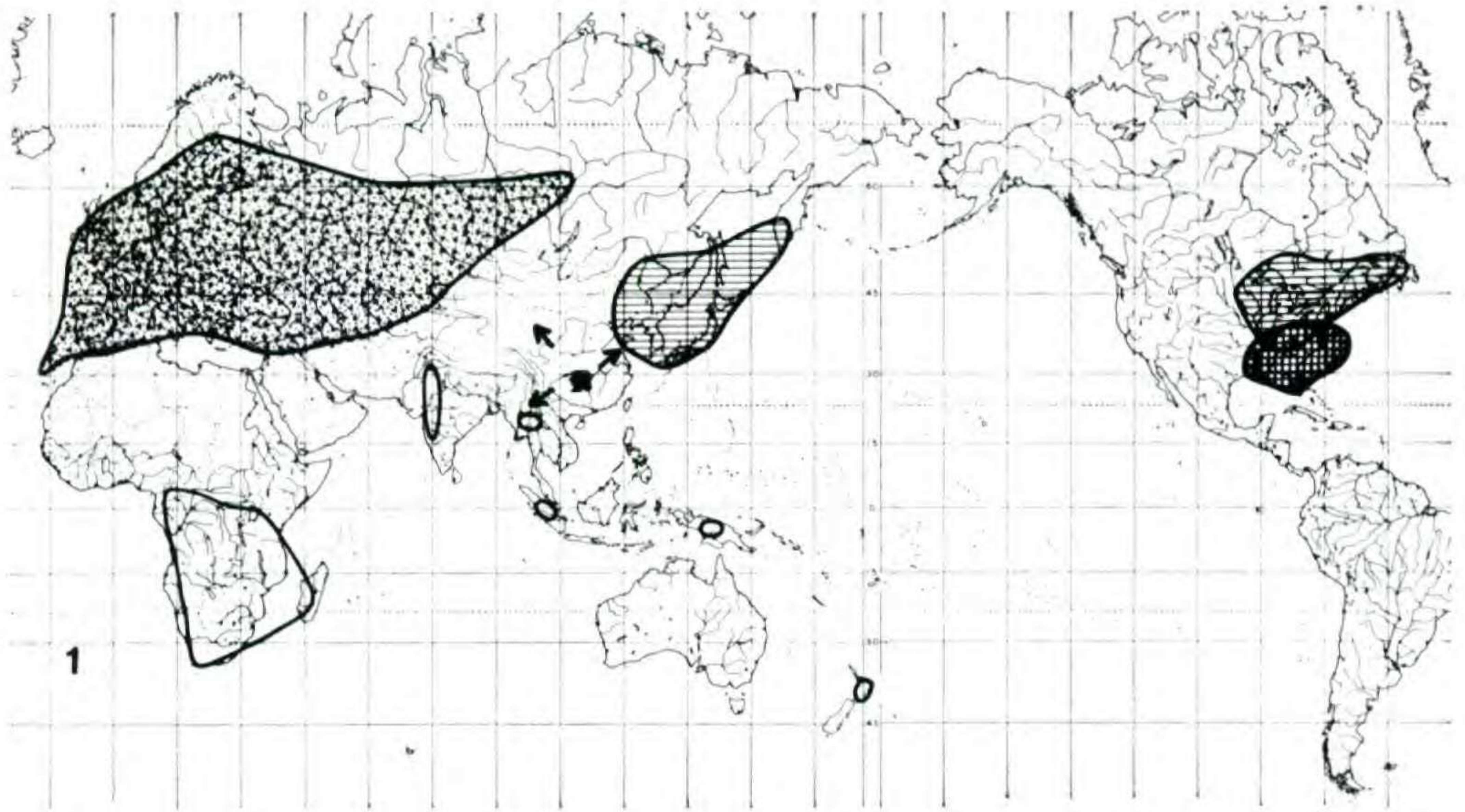


Fig. 1. Generalized geographic ranges of the varieties of *Thelypteris paustris* adapted from Hultén (1962): var. *palustris*, stippled; var. *pubescens*, horizontal lines; var. *Haleana*, vertical and horizontal lines; var. *squamigera*, ellipses. The star represents a proposed central region of the complex with the arrows indicating radiation from it.

*squamigera*, for example, may reflect earlier range expansion and differentiation in that variety. The absence of plants in western North America and in much of southeast Asia may, in part, reflect the effects of Pleistocene glaciation in these regions. The wide geographic range may be accounted for in the evolutionary adaptations of the plants. Especially in the north, the range is correlated with the widespread regions of cool, wet climates with a dormant season.

The close relationship between the geographic elements of *Thelypteris palustris* in eastern North America and northeastern Asia is a familiar one in ferns. In these two regions, the same or vicarious species occur in *Camptosorus*, *Dennstaedtia*, *Diplazium* and others reported by Tryon (1970). Similar floristic relationships are shown by Li (1971) for many genera of flowering plants. The relationships, indicated by the spores, between the eastern North American and eastern Asian var. *pubescens* and var. *Haleana* in southeastern North America are of particular in-

terest and deserve additional attention. On the basis of the more modified fertile leaves with simple veins, collections from northeastern Asia are best placed with var. *pubescens*. However, the more prominently sculptured perine layer in the Japanese specimens show marked similarity to spores of var. *Haleana*. There are some differences between the spores from the western part of the range of var. *pubescens* in the United States and those of the east. The western plants, which also differ in scale and vein characters, may reflect relic populations of a less derived type or possibly represent more recent northward range expansion of populations of var. *Haleana*.

#### CONCLUSION

The survey of spores of *Thelypteris palustris* has shown new details of the wall structure and has revealed wide variation in the form of the perine layer. The variation is too great to permit species characterization such as is usually possible in ferns. These different patterns are attributed to adaptations of the plants as they have become dispersed over a broad geographic range.

#### LITERATURE CITED

- CHING, R. C. 1963. A reclassification of the family Thelypteridaceae from the mainland of Asia, *Acta Phytotax. Sinica* 8: 289-335.
- FERNALD, M. L. 1929. A study of *Thelypteris palustris*. *Rhodora* 31: 27-36.
- HARRIS, W. F. 1955. A manual of the spores of New Zealand Pteridophyta. *Bull. New Zealand Dept. Scientific and Industrial Research* 116: 1-186.
- HENNIPMAN, E. 1970. Electron and light microscopical observations on the perine of the spores of some *Bolbitis* species (Filicales). *Acta Bot. Neerl.* 19: 671-679.
- HESLOP-HARRISON, J. 1968. Pollen wall development. *Science* 161: 230-237.
- HOLTUM, R. E., U. SEN and D. MITTRA. 1970. Studies in the family Thelypteridaceae II. A comparative study of the type-species of *Thelypteris* Schmidel, *Cyclosorus* Link, and *Ampelopteris* Kunze. *Blumea* 18: 195-215.
- HULTÉN, E. 1962. The circumpolar plants I. Vascular Cryptogams, Conifers, Monocotyledons. *Kungl. Svensk. Vet. Handl.* 8: 1-275.



- LI, H. 1971. Floristic relationships between eastern Asia and eastern North America. Morris Arboretum Monographs. Philadelphia, Pa.
- MORTON, C. V. 1967. Studies of fern types I. Contrib. U. S. Nat. Herb. 38: 29-83.
- PETTITT, J. M. 1966. Exine structure in some fossil and recent spores and pollen as revealed by light and electron microscopy. Bull. Brit. Mus. (Nat. Hist.) Geology 13: 221-257.
- SORSA, P. 1964. Studies on the spore morphology of Fennoscandian fern species. Ann. Bot. Fenn. 1: 179-201.
- TRYON, ROLLA. 1969. Taxonomic problems on the geography of North American ferns. Bioscience 19: 790-795.

GRAY HERBARIUM

HARVARD UNIVERSITY

CAMBRIDGE, MASSACHUSETTS 02138

**PEUCEDANUM PALUSTRE AGAIN IN MASSACHUSETTS:** On August 4, 1970 I noticed at the Great Meadows Wildlife Refuge in Concord, Massachusetts, a single large plant of an umbellifer at the edge of impounded water. I was unable to find it in the older manuals but was eventually able to identify it as *Peucedanum palustre* (L.) Moench. from Seymour's Flora of New England where it was based on one specimen in the New England Botanical Club's herbarium. This specimen collected in West Newbury, Essex County by S. K. Harris July 9, 1958 is apparently the first and only North American record of the species. (Rhodora 61: 181). On August 15, 1970 I noticed about three dozen plants of this same species covering an area of about 20 × 40 feet in a damp meadow with *Eupatorium dubium*, *Solidago graminifolia*, *Sambucus canadensis* and *Onoclea sensibilis* beside a brook that flows into the Concord River a mile upstream from the Wildlife Refuge. The colloquial names are Marsh Hog's Fennel and Milk Parsley, the latter appropriate because of the milky sap that bleeds out when the stem is cut. The appearance of this colony over thirty miles from the discovery twelve years ago makes it seem probable that this species can be found in other locations in eastern Massachusetts, but has been overlooked by the